



Supplemental Material to:

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Variability of non-symbiotic and truncated hemoglobin genes from the genome of cultivated monocots

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Figure S1
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	<i>H. vulgare</i> nsHb-I	<i>T. aestivum</i> nsHb-I	<i>P. virgatum</i> nsHb1-I	<i>P. virgatum</i> nsHb2-I	<i>O. glaberrima</i> nsHb1-I	<i>O. sativa japonica</i> nsHb2-I	<i>O. sativa indica</i> nsHb2-I	<i>O. sativa japonica</i> nsHb3-I	<i>O. sativa indica</i> nsHb3-I	<i>O. sativa japonica</i> nsHb4-I	<i>O. sativa indica</i> nsHb4-I	<i>O. sativa japonica</i> nsHb5-I	<i>O. sativa indica</i> nsHb5-II	<i>B. distachyon</i> nsHb1-II	<i>S. italica</i> nsHb1-II	<i>Z. mays</i> nsHb1-II	<i>S. bicolor</i> nsHb1-II	<i>P. virgatum</i> nsHb1-II	<i>H. vulgare</i> nsHb1-II							
<i>H. vulgare</i> nsHb-I	98.1	85.8	82.5	78.4	79.3	79.3	79.3	72.8	72.8	78.9	71.9	71.9	82.4	86.1	86.6	86.9	59.8	57.9	61.1	53.9	50.3	52.4	56.4	60.2		
<i>T. aestivum</i> nsHb-I	99.4	84	80.7	84.8	77.5	77.5	77.5	71	71	77.7	77.7	70.7	82.4	84.8	84.8	86.3	58.5	56.7	59.9	52.7	49.2	51.3	55.2	59		
<i>P. virgatum</i> nsHb1-I	92	91.4	94.6	83.6	84.6	84.6	84.6	73.4	73.4	80.7	80.7	73.7	73.7	89.1	92.1	94.5	80.4	61.6	61	61.1	56.3	52.3	54.5	57.7	60.8	
<i>P. virgatum</i> nsHb2-I	89.2	88.6	95.2	83.6	83.6	83.6	83.6	73.7	73.7	79.2	79.2	73.4	73.4	87.4	91	93.4	80.6	60.1	59.5	59.6	55	51.2	53.4	56.3	59.4	
<i>O. glaberrima</i> nsHb-I	85.4	76.6	87.7	90.1	98.8	98.8	98.8	80.2	80.2	90.1	90.1	74	74	79.5	84.8	84.8	79.7	57.2	57.2	58	52.8	49	51	54.1	57.1	
<i>O. rufipogon</i> nsHb-I	86.4	85.8	88.8	88.9	98.8	100	100	81.2	81.2	91.1	91.1	74.9	74.9	80.5	85.8	85.8	80.6	57.9	57.3	58	52.9	49	51	54.1	57.2	
<i>O. sativa japonica</i> nsHb2-I	86.4	85.8	88.8	88.9	98.8	100	100	81.2	81.2	91.1	91.1	74.9	74.9	80.5	85.8	85.8	80.6	57.9	57.3	58	52.9	49	51	54.1	57.2	
<i>O. sativa indica</i> nsHb2-I	86.4	85.8	88.8	88.9	98.8	100	100	81.2	81.2	91.1	91.1	74.9	74.9	80.5	85.8	85.8	80.6	57.9	57.3	58	52.9	49	51	54.1	57.2	
<i>O. sativa japonica</i> nsHb3-I	84	83.4	83.4	82.5	86	87.1	87.1	87.1	100	100	78.8	78.8	86	86	71.6	74.7	74.6	73.5	55	55.6	57.2	50.9	47.8	50.8	61.2	55.8
<i>O. sativa indica</i> nsHb3-I	84	83.4	83.4	82.5	86	87.1	87.1	87.1	100	100	78.8	78.8	86	86	71.6	74.7	74.6	73.5	55	55.6	57.2	50.9	47.8	50.8	61.2	55.8
<i>O. sativa japonica</i> nsHb1-I	84	83.4	83.4	82.5	86	87.1	87.1	87.1	100	100	78.8	78.8	86	86	71.6	74.7	74.6	73.5	55	55.6	57.2	50.9	47.8	50.8	61.2	55.8
<i>O. sativa indica</i> nsHb1-I	84	83.4	83.4	82.5	86	87.1	87.1	87.1	100	100	78.8	78.8	86	86	71.6	74.7	74.6	73.5	55	55.6	57.2	50.9	47.8	50.8	61.2	55.8
<i>O. sativa japonica</i> nsHb4-I	87.3	88.3	87.3	86.9	92.4	93.5	93.5	94.7	84.7	84.7	100	100	73.8	73.8	78.9	83.1	81.9	74.6	54.9	59.5	59.1	55.6	50.7	52.8	56.3	59.4
<i>O. sativa indica</i> nsHb4-I	87.3	88.3	87.3	86.9	92.4	93.5	93.5	94.7	84.7	84.7	100	100	73.8	73.8	78.9	83.1	81.9	74.6	54.9	59.5	59.1	55.6	50.7	52.8	56.3	59.4
<i>O. sativa japonica</i> nsHb4-I	83.24	82.6	83.2	82.8	80.3	81.3	81.3	81.3	90.6	90.6	82.7	82.7	100	72.5	73.7	74.3	71.8	53.3	52.7	54.1	48.2	45.5	47.9	48.8	53.8	
<i>O. sativa indica</i> nsHb4-I	83.4	82.6	83.2	82.8	80.3	81.3	81.3	81.3	90.6	90.6	82.7	82.7	100	72.5	73.7	74.3	71.8	53.3	52.7	54.1	48.2	45.5	47.9	48.8	53.8	
<i>Z. mays</i> nsHb-I	89.1	89.7	94.5	94	87.7	88.8	88.8	88.8	83.4	83.4	88	88	83.2	83.2	94.5	90.3	79.8	57.5	57.5	57.6	53.5	50.5	51.6	55.4	58.6	
<i>S. bicolor</i> nsHb-I	91.5	90.9	94.5	94	88.9	89.9	89.9	89.9	85.3	85.3	89.2	89.2	84.4	84.4	98.8	95.2	81.7	59.3	59.3	59.4	54.7	51	63.1	56	59.2	
<i>S. italica</i> nsHb-I	96.1	91.5	96.3	95.8	89.5	90.5	90.5	90.5	84.6	84.6	88.6	88.6	84.4	84.4	95.8	97	82.1	60.8	60.8	60.9	56.2	52.2	54.5	57.6	60.7	
<i>B. distachyon</i> nsHb-I	92.3	91.7	88.7	88.2	88.4	89.4	89.4	89.4	84.7	84.7	84.6	84.6	82.9	82.9	89.3	88.8	89.9	57.1	56.5	59.5	53.2	48.8	52.3	55.6	59.3	
<i>O. sativa indica</i> nsHb5-II	72	72	71.3	69	67.6	68.4	68.4	68.4	67.8	67.8	70.2	70.2	66.3	66.3	68.9	70.7	71.1	70	97.2	79.2	82.3	64.1	70.2	85.5	80.4	
<i>O. sativa japonica</i> nsHb5-II	71.3	71.3	71.3	69	67.6	68.4	68.4	68.4	67.8	67.8	70.2	70.2	66.3	66.3	68.9	70.7	71.1	69.4	100	79.9	83.7	64.6	71.3	86.9	80.4	
<i>B. distachyon</i> nsHb-II	74.3	74.3	72.5	70.2	68.2	69	69	69	69.4	69.4	70.8	70.8	69.4	69.4	70.6	71.8	72.2	71.7	86.8	86.2	76.7	69.9	74.6	79.2	91.8	
<i>S. italica</i> nsHb-II	66.5	72.5	66.5	64.3	63.6	64.4	64.4	64.4	63.7	63.7	66.1	66.1	63.1	63.1	63.5	65.9	66.3	65.9	89.8	89.8	81.1	69.1	73.5	93.8	79.1	
<i>Z. mays</i> nsHb-II	60.3	60.3	61.3	59.6	59.1	57.7	57.7	57.7	59.5	59.5	60.6	60.6	57.9	57.9	58.9	60.4	61.2	60	69.3	69.3	73.1	71.2	79.5	69.6	73.4	
<i>S. bicolor</i> nsHb-II	64	64	64.6	65.7	61.6	62.2	62.2	62.2	62.1	62.1	63.7	63.7	60.9	60.9	62.5	64.1	64.4	63.6	75.1	75.1	78.5	76.2	82.6	74.6	77.9	
<i>P. virgatum</i> nsHb-II	69.3	69.3	68.1	65.9	65.1	65.9	65.9	65.9	64.1	64.1	67.7	67.7	63.1	63.1	65.7	67.5	67.9	68.6	93.1	93.1	83	94.5	71.2	76.2	81.6	
<i>H. vulgare</i> nsHb-II	74.7	74.7	72.9	70.6	69.7	70.5	70.5	69.8	69.8	72.4	72.4	69.2	70.4	72.2	72.6	73.8	86.1	86.1	96.2	83.5	75	81.2	84.8	81.6		

(A)

	<i>H. vulgare</i>	<i>T. aestivum A</i>	<i>T. aestivum B</i>	<i>B. distachyon</i>	<i>S. italica</i>	<i>P. virgatum</i>	<i>S. bicolor</i>	<i>O. sativa japonica</i>	<i>Z. mays</i>	<i>O. sativa indica</i>
<i>H. vulgare</i>	96.5	98.2	92.4	94.2	86	88.9	88.9	84.3	84.3	84.3
<i>T. aestivum A</i>	99.4	97.1	91.8	87.1	84.9	84.7	87.7	84.3	84.3	84.3
<i>T. aestivum B</i>	99.4	98.8	93	88.9	86.6	89.5	89.5	84.3	84.3	84.3
<i>B. distachyon</i>	96.5	95.9	95.9	87.1	84.3	86.5	86.5	82.6	82.6	82.6
<i>S. italica</i>	88.3	93.6	93.6	92.4	94.8	95.3	94.7	86	86	86
<i>P. virgatum</i>	91.3	90.7	90.7	89.5	95.3	92.4	92.4	82.7	82.7	82.7
<i>S. bicolor</i>	95.3	94.7	94.7	92.4	96.5	94.2	97.1	85	85	85
<i>Z. mays</i>	93.6	93	93	91.2	94.7	92.4	98.2	84.4	84.4	84.4
<i>O. sativa japonica</i>	89.5	90.1	89.5	88.4	91.3	87.3	91.3	89.6	100	100
<i>O. sativa indica</i>	89.5	90.1	89.5	88.4	91.3	87.3	91.3	89.6	100	100

(B)

Figure S2
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	<i>O. rufipogon nshb-I</i>	<i>O. glaberrima nshb-I</i>	<i>O. sativa japonica nshb2-I</i>	<i>O. sativa indica nshb2-I</i>	<i>O. sativa japonica nshb1-I</i>	<i>O. sativa indica nshb1-I</i>	<i>O. sativa japonica nshb3-I</i>	<i>O. sativa indica nshb3-I</i>	<i>O. sativa japonica nshb4-I</i>	<i>O. sativa indica nshb4-I</i>	<i>T. aestivum nshb4-I</i>	<i>H. vulgare nshb-I</i>	<i>P. virgatum nshb1-I</i>	<i>P. virgatum nshb2-I</i>	<i>Z. mays nshb-I</i>	<i>S. bicolor nshb-I</i>	<i>B. distachyon nshb-I</i>	<i>O. sativa japonica nshb5-II</i>	<i>O. sativa indica nshb5-II</i>	<i>B. distachyon nshb-II</i>	<i>S. italica nshb-II</i>	<i>Z. mays nshb-II</i>	<i>S. bicolor nshb-II</i>	<i>P. virgatum nshb-II</i>	<i>H. vulgare nshb-II</i>		
<i>O. rufipogon nshb-I</i>	100	100	100	93	93	89	89	84	84	82	84	85	85	84	85	86	83	62	62	63	63	55	58	61	68		
<i>O. glaberrima nshb-I</i>	0	0	100	93	93	89	89	84	84	82	84	85	85	86	86	83	62	62	63	63	55	58	61	68			
<i>O. sativa japonica nshb2-I</i>	0	0	0	93	93	89	89	84	84	82	84	85	85	86	86	83	62	62	63	63	55	58	61	68			
<i>O. sativa indica nshb2-I</i>	0	0	0	93	93	89	89	84	84	82	84	85	85	86	86	83	62	62	63	63	55	58	61	68			
<i>O. sativa japonica nshb1-I</i>	38	38	38	38	38	100	86	86	83	83	83	84	84	83	83	85	79	65	65	64	64	57	60	65	70		
<i>O. sativa indica nshb1-I</i>	38	38	38	38	38	0	85	85	83	83	83	84	84	83	83	85	79	65	65	64	64	57	60	65	70		
<i>O. sativa japonica nshb3-I</i>	59	59	59	59	59	72	72	0	89	89	89	80	82	83	82	81	81	82	80	62	63	63	63	56	58	61	68
<i>O. sativa indica nshb3-I</i>	59	59	59	59	59	78	78	0	89	89	89	80	82	83	82	81	81	82	80	62	63	63	63	56	58	61	68
<i>O. sativa japonica nshb4-I</i>	82	82	82	82	82	87	87	56	56	100	80	79	81	81	81	81	81	76	60	60	60	60	64	57	60	67	
<i>O. sativa indica nshb4-I</i>	82	82	82	82	82	84	84	101	101	101	0	80	79	81	81	81	81	76	60	60	60	60	64	57	60	67	
<i>T. aestivum nshb-I</i>	84	84	84	87	87	101	101	104	104	104	96	85	85	84	85	86	86	86	64	64	63	63	55	59	62	69	
<i>H. vulgare nshb-I</i>	83	83	83	84	84	94	94	107	107	107	22	86	85	86	86	86	87	65	65	64	64	64	56	63	69		
<i>P. virgatum nshb1-I</i>	79	79	79	80	80	86	86	96	96	96	76	68	97	88	89	93	79	66	66	68	68	58	61	66	73		
<i>P. virgatum nshb2-I</i>	76	76	76	76	80	80	92	92	94	94	74	75	17	90	91	95	81	65	64	66	66	58	61	65	72		
<i>Z. mays nshb-I</i>	84	84	84	85	85	100	100	98	98	98	78	71	63	49	95	91	81	63	62	63	63	56	59	63	69		
<i>S. bicolor nshb-I</i>	76	76	76	89	89	96	96	97	97	97	75	68	57	46	26	93	81	63	63	63	63	56	60	62	69		
<i>S. italica nshb-I</i>	73	73	73	73	78	78	91	91	97	97	71	68	34	27	45	36	81	65	65	65	65	57	61	64	71		
<i>B. distachyon nshb-I</i>	90	90	90	90	112	112	102	102	127	127	70	66	107	99	100	100	97	60	61	62	62	54	59	62	66		
<i>O. sativa indica nshb5-II</i>	198	198	198	181	181	199	199	207	207	182	177	174	180	191	190	183	210	99	73	73	73	54	69	57	80		
<i>O. sativa japonica nshb5-II</i>	198	198	198	179	179	197	197	207	207	182	185	174	183	196	195	182	208	4	74	74	65	69	87	80			
<i>B. distachyon nshb-II</i>	197	197	197	197	192	192	196	196	210	210	192	185	164	175	197	193	186	200	128	126	100	70	74	81	81		
<i>S. italica nshb-II</i>	197	197	197	197	192	192	196	196	210	210	192	185	164	175	197	193	186	200	128	126	0	70	74	81	81		
<i>Z. mays nshb-II</i>	287	287	287	287	266	266	279	279	294	294	285	278	259	261	273	275	271	298	206	204	175	175	86	69	71		
<i>S. bicolor nshb-II</i>	262	262	262	241	241	253	253	282	282	282	245	233	230	233	246	242	232	244	170	168	145	145	85	74	75		
<i>P. virgatum nshb-II</i>	210	210	210	210	180	180	209	209	210	210	200	191	172	184	195	203	192	202	87	55	90	90	178	141	81		
<i>H. vulgare nshb-II</i>	172	172	172	172	155	155	169	169	175	175	161	163	138	149	163	164	156	180	98	98	93	93	171	138	90		

(A)

	<i>H. vulgare</i>	<i>T. aestivum A</i>	<i>T. aestivum B</i>	<i>B. distachyon</i>	<i>O. sativa japonica</i>	<i>O. sativa indica</i>	<i>S. italica</i>	<i>P. virgatum</i>	<i>S. bicolor</i>	<i>Z. mays</i>
<i>H. vulgare</i>	96	97	92	87	87	89	87	87	87	85
<i>T. aestivum A</i>	19	97	92	87	87	88	86	87	87	85
<i>T. aestivum B</i>	14	15	93	88	88	89	87	87	88	86
<i>B. distachyon</i>	41	40	37	88	88	87	87	87	86	84
<i>O. sativa japonica</i>	67	66	64	63	100	86	86	86	86	84
<i>O. sativa indica</i>	67	66	64	63	0	86	86	86	86	84
<i>S. italica</i>	59	60	57	68	81	81	94	94	93	92
<i>P. virgatum</i>	67	71	68	70	76	76	29	29	92	90
<i>S. bicolor</i>	69	68	65	75	75	75	37	41	41	96
<i>Z. mays</i>	78	77	74	84	86	86	43	50	19	

(B)

Figure S1. Distance matrix for cultivated monocot nsHb (A) and tHb (B) proteins.

Identity and similarity percentages are shown above and below the diagonal, respectively. Quadrants in matrix (A) correspond to pairwise sequence alignments among nsHbs-I (upper left quadrant), nsHbs-II (lower right quadrant) and nsHbs-I vs. nsHbs-II (upper right and lower left quadrants). Higher and lower values are shown in blue and red colors, respectively.

Figure S2. Distance matrix for coding regions from cultivated monocot *nshb* (A)

and *thb* (B) genes. Values for base substitution and identity percentages in pairwise nucleotide sequence alignments are shown below and above the diagonal, respectively. Quadrants in matrix (A) correspond to pairwise sequence alignments among *nshbs*-I (upper left quadrant), *nshbs*-II (lower right quadrant) and *nshbs*-I vs. *nshbs*-II (upper right and lower left quadrants). Higher and lower values are shown in blue and red colors, respectively.

Table S1. Binomial and database accession number for cultivated monocot (clade I and II) nsHbs.⁸

Monocot binomial	Protein/Gene	Clade	Accession no.	Database
<i>Brachypodium distachyon</i>	nsHb	I	Bradi1g69320.1	Phytozome
	nsHb	II	Bradi2g19690.1	Phytozome
<i>Hordeum vulgare</i>	nsHb	I	AAB70097.1	GenBank
	nsHb	II	NIASHv3120P12	Barley full-length cDNA database
<i>Oryza glaberrima</i>	nsHb	I	AC210470.1 (position 19510-20342)	GenBank
<i>Oryza rufipogon</i>	nsHb	I	CU406294.1	GenBank
<i>Oryza sativa</i> var. indica	nsHb 1	I	AAC49882.1	GenBank
	nsHb 2	I	AAC49881.1	GenBank
	nsHb 3	I	EAY89159.1	GenBank
	nsHb 4	I	EAY89160.1	GenBank
	nsHb 5	II	ABN45744.1	GenBank
<i>Oryza sativa</i> var. japonica	nsHb 1	I	AAK72229.1	GenBank
	nsHb 2	I	AAK72228.1	GenBank
	nsHb 3	I	AAK72230.1	GenBank
	nsHb 4	I	AAK72231.1	GenBank
	nsHb 5	II	AAT01321.1	GenBank
<i>Panicum virgatum</i>	nsHb 1	I	Pavirv00001133m	Phytozome
	nsHb 2	I	Pavirv00043867m	Phytozome
	nsHb	II	sg0.contig01192:20077..21247	Phytozome
<i>Setaria italica</i>	nsHb	I	Si037496m (aa 47..210)	Phytozome
	nsHb	II	Si023403m	Phytozome
<i>Sorghum bicolor</i>	nsHb	I	Sb01g042260.1	Phytozome
	nsHb	II	Sb09g025730.2	Phytozome
<i>Triticum aestivum</i>	nsHb	I	AAN85432.1	Genbank
<i>Zea mays</i> ssp. <i>mays</i>	nsHb	I	GRMZM2G067402_T02	Phytozome
	nsHb	II	GRMZM2G168898_T01	Phytozome

Table S2. Binomial and database accession number for cultivated monocot tHbs.

Monocot binomial	Protein/Gene	Accession no.	Database
<i>Brachypodium distachyon</i>	tHb	Bradi1g37100.1	Phytozome
<i>Hordeum vulgare</i>	tHb	AAK55410.1	GenBank
<i>Oryza sativa</i> var. <i>indica</i>	tHb	EEC80902.1	GenBank
<i>Oryza sativa</i> var. <i>japonica</i>	tHb	NP_001057972.1	GenBank
<i>Panicum virgatum</i>	tHb	Pavirv00015565m.g	Phytozome
<i>Setaria italica</i>	tHb	Si007381m	Phytozome
<i>Sorghum bicolor</i>	tHb	Sb10g022990.1	Phytozome
<i>Triticum aestivum</i>	tHb A	ACH86231.1	GenBank
	tHb B	ACH86230.1	GenBank
<i>Zea mays</i> ssp. <i>mays</i>	tHb	GRMZM2G155868_T01	Phytozome